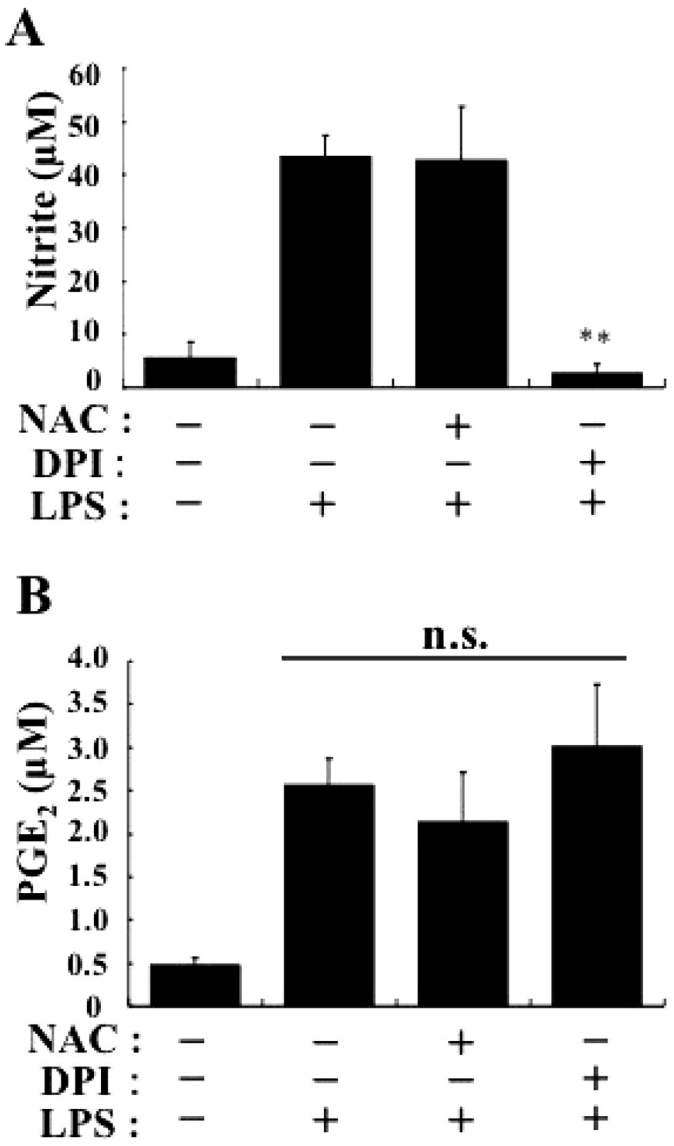

508
MARKERS OF ATHEROSCLEROSIS IN RELATION TO PRESENCE AND PROGRESSION OF KNEE OSTEOARTHRITIS: THE ROTTERDAM STUDY

T.A. Hoeven, M. Kavousi, M.A. Ikram, J.B. van Meurs, P.J. Bindels, A. Hofman, O.H. Franco, S.M. Bierma-Zeinstra. *Erasmus Univ. Med. Ctr., Rotterdam, Netherlands*

Purpose: Several observational studies have found an association between subclinical measures of atherosclerosis and osteoarthritis (OA) of the hands and knees, predominantly among women. Different mechanisms have been suggested to explain the potential relation between atherosclerosis and OA; systemic low-grade inflammation caused by visceral adipose tissue is particularly mentioned and may consequently highlight a route to improve prevention and treatment of atherosclerosis and OA. However, the reported associations between subclinical measures of atherosclerosis and OA were modest in effect size, derived mainly from cross-sectional studies and generally attenuated after adjustment for cardiovascular risk factors. Furthermore, previous results were inconsistent for the different imaging markers of atherosclerosis. In previous work, we found no relation between peripheral measurements of atherosclerosis (including carotid intima-media-thickness or carotid plaque) and progression of knee, hand, or hip OA in a large sample of a prospective cohort study. Hence, it remains unclear whether atherosclerosis and OA are related or whether they are simply independent disorders with shared risk factors. Coronary artery calcification (CAC) is considered a late stage pathognomonic feature of coronary atherosclerosis. CD40L, VCAM-1, and VEG-f are serum biomarkers that reflect initial inflammatory stages of vascular wall damage in the ischemic cascade. In the vascular endothelium, VEG-f is a protein that stimulates angiogenesis, CD-40L and VCAM-1 are molecules that initiate coagulation and immune responses. We therefore investigated the association between these markers of early and late atherosclerosis and presence and progression of knee OA, a possible cardiometabolic phenotype of OA, in a large population-based cohort study.

Methods: The analyses on prevalence of knee OA were performed in 3,465 participants from the prospective population-based Rotterdam Study (mean age 73.1 years, 61% women). Data on coronary artery calcification (CAC) were available for 1,669 participants and plasma levels of CD40L, VCAM-1, and VEG-f in 975. For the analyses on progression of knee OA, data on CAC was available for 979 participants (17% progressors), and 246 participants (20% progressors) had plasma levels of CD40L, VCAM-1, and VEG-f. We scored radiographs of the knee with the Kellgren-Lawrence (K&L) score for osteoarthritis (knee OA present with a K&L graded score greater or equal to 2) at baseline and follow-up (average follow-up time 4.5 years (±0.5)). Overall progression of knee OA was defined as the combination of the incidence and the progression of existing OA at baseline and was considered present if the K&L score increased 1 grade between baseline and follow-up visit. After stratification by gender, multivariate logistic regression models with generalized estimated equations on knee level were used to calculate odds ratios (95% confidence intervals) for prevalence and progression of knee OA per each SD increase in marker levels.

Results: Within the study population, 18% had radiographic knee OA, 11% of the men, 23% of the women. CAC and VEG-f were not associated with prevalent knee OA. Among women, CD40L (adjusted odds ratio (aOR) 1.31 (1.12 to 1.56)) and VCAM-1 (aOR 1.31 (1.08 to 1.59)) were associated with prevalent knee OA (table). No associations with progression were found in women. In men, too few progressors were available to assess associations.

Table. Markers of atherosclerosis in relation to prevalent knee OA, stratified by gender

	Knee OA (Women) OR (95% CI)*	Knee OA (Men) OR (95% CI)*
Coronary artery calcification	1.11 (0.95–1.30)	1.11 (0.86–1.43)
CD40L	1.32 (1.12–1.56)**	1.05 (0.80–1.37)
VCAM-1	1.31 (1.08–1.59)**	1.08 (0.82–1.42)
VEG-f	1.08 (0.93–1.24)	1.07 (0.90–1.28)

Conclusions: In this population-based study, coronary artery calcification and VEG-f were not associated with presence or progression of knee OA. However, plasma levels of CD40L and VCAM-1 were higher in women with knee OA and not in men. This might reflect an association between early atherosclerosis and knee OA through low-grade systemic inflammation in women.

509
MECHANISMS INVOLVED IN INHIBITION OF INFLAMMATION IN THP-1 CELLS BY THE HEXADECYLAMIDE DERIVATIVE OF HYALURONIC ACID.

F. Oliviero †, A. Scanu †, R. Ramonda †, P. Frallonardo †, P. Sfriso †, J. Dayer ‡, L. Punzi †. † *Rheumatology Unit, Univ. of Padova, Padova, Italy;* ‡ *Faculty of Med., Geneva, Switzerland*

Purpose: Intra-articular injections of hyaluronic acid are widely used in the treatment of inflammatory and degenerative joint diseases. The immune regulation exerted by hyaluronic acid is modulated by its interaction with different receptors including CD44 and the toll-like receptors 2 and 4. A novel hexadecylamide derivative of hyaluronic acid (HA), HYADD®4, has recently been tested in animal models of osteoarthritis, showing both anti-inflammatory and anabolic effects. The purpose of this study is to investigate the possible mechanisms involved on the effect of unmodified and hexadecylamide derivative

(commercially, Hymovis®) of HA on some inflammatory aspects of the osteoarthritis process reproduced in vitro.
Methods: The human leukemic monocytic cell line THP-1 was treated with CPP crystals (0.025 mg/ml) or LPS (500 ng/ml) and cultured for 24 hours with either simultaneous or 30' delayed addition of fresh media containing 0.5 mg/mL unmodified HA (HN 500-730 kDa, HX >1,500 kDa) or the hexadecylamide derivative of HA (Hymovis®) (HS), all supplied by Fidia Farmaceutici SpA. In some experiments, cells were primed for 3 h with PMA at 300 ng/ml or pre-treated for 2 h with CD44 function-blocking monoclonal antibody (10 μg/ml) or an isotype control prior to stimulation. The levels of IL-1ß and IL-8 were determined in the culture supernatants by ELISA assays. The effect of HA on the phagocytic capacity of THP-1 was evaluated by ordinary/polarized light microscopy. The scavenger effect of HA on cytokines was determined through the indirect quantification of the binding of these proteins to the chemical structure of HA. The expression of CD44 on THP-1 cells was assessed by fluorescence-activated cell sorter (FACS) analysis.
Results: THP-1 cells produce high basal levels of both IL-1ß and IL-8 which further increase after 24 h treatment with CPP crystals. The addition of 0.5 mg/mL of HA along with the stimulus lead to about the 60% inhibition of cytokine release using HS with compared to HN and HX. Any scavenger effect of HS due to the binding of IL-1ß and IL-8 to its chemical structure was ruled out. HS displayed a moderate inhibitory effect on crystal phagocytosis. All three derivatives showed a strong inhibitory effect on LPS-induced IL-1ß and IL-8 production when added simultaneously with the stimulus, but only HS was able to block inflammation once started. This effect was confirmed in presence of CPP crystals. THP-1 constitutively express CD44 receptor as evidenced by FACS analysis. Nevertheless, the incubation with the anti-CD44 antibody did not alter HS effect on THP-1 inflammatory response.
Conclusions: The results of this study show that the hexadecylamide derivative of HA is able to suppress IL-1ß and IL-8 production after CPP crystal and LPS stimulation of monocytes. The fact that 1) HS, but not unmodified HA, acts also once inflammation is triggered, 2) it do not exhibit scavenger effect and 3) its action is not stimulus-specific, allows us to hypothesize that the anti-inflammatory activity of HS could be modulated by its interaction with components/receptors of cell surface other than CD44, at least in this model.

510
CIRCULATING C-REACTIVE PROTEIN IN OSTEOARTHRITIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

X. Jin †, J. Beguerie ‡, W. Zhang §, L. Blizzard †, P. Otahal †, G. Jones †, C. Ding ‖,†. † *Menzies Res. Inst. Tasmania, Hobart, Australia;* ‡ *Dept. of Dermatology, Austral Univ. Hosp., Buenos Aires, Argentina;* § *Academic Rheumatology, Univ. of Nottingham, Nottingham, United Kingdom;* ‖ *Dept. of Epidemiology and Preventive Med., Monash Univ., Melbourne, Australia*

Purpose: There is emerging evidence that the development and progression of osteoarthritis (OA) is associated with inflammation. C-reactive protein (CRP), a systemic marker for inflammation, may be elevated in OA patients but the evidence is conflicting. We systematically reviewed the literature for the relationship between serum CRP levels measured by a high sensitivity method (hs-CRP) and OA, as well as the correlation between circulating CRP levels and OA phenotypes.
Methods: MEDLINE, EMBASE and CINAHL databases were systematically searched from January 1992 to December 2012. Studies were included when they met the inclusion criteria and data from studies were extracted. Two independent reviewers assessed study quality using a modified Newcastle-Ottawa Quality Assessment Scale (NOQAS). Meta-analyses were performed to pool available data from included studies. MEDLINE, EMBASE and CINAHL databases were systematically searched from January 1992 to December 2012. Studies were included when they met the inclusion criteria and data from studies were extracted. Two independent reviewers assessed study quality using a modified Newcastle-Ottawa Quality Assessment Scale (NOQAS). Meta-analyses were performed to pool available data from included studies.
Results: Ten case-control, 15 cross-sectional, 4 longitudinal studies and 3 clinical trials met the criteria and were thus included. Data of 17,090 participants (6,440 OA cases and 10,650 controls) were extracted. The average methodological quality across included studies was satisfactory. Except for one study showing no difference between OA and controls, all other studies revealed that circulating levels of hs-CRP were higher in OA patient than in healthy controls (Fig. 1). The pooled mean difference showed that hs-CRP level was significantly higher in OA than in controls, with an average increase in value of 1.19 mg/l (95% CI 0.64 to 1.73, $p < 0.001$). The elevation in serum hs-CRP levels was greater in hip OA than in knee OA, 3.37 mg/l (95% CI 0.60 to 6.13) compared to 1.15 mg/l (95% CI 0.18 to 2.12). The links between serum hs-CRP levels and knee radiographic OA were investigated in four studies. The pooled correlation coefficient showed that the link between serum hs-CRP levels and knee radiographic OA was weak and it was not statistically significant ($r = 0.11$, 95% CI −0.03 to 0.26, $p = 0.13$). The correlation coefficients between hs-CRP levels and pain in OA patients were available in six studies. The pooled result of the meta-analysis showed that there was a weak but statistically significant correlation between hs-CRP levels and pain scale score ($r = 0.14$, 95% CI 0.08 to 0.20, $p < 0.001$). There was no significant heterogeneity observed across the studies ($Chi2 = 4.12$, $p = 0.39$; $I2 = 3\%$). Two studies reported the correlation coefficient of hs-CRP with physical function. The results from both studies were consistent with each other, indicating a correlation between increased hs-CRP levels and worsening physical function. The pooled correlation coefficient was statistically significant ($r = 0.26$, 95% CI 0.13 to 0.39, $p < 0.001$)
Conclusions: OA is chronic joint disorder with low grade systemic inflammation as reflected by increased serum hs-CRP levels. Low-grade systemic inflammation may play a greater role in symptoms rather than radiographic changes in OA.

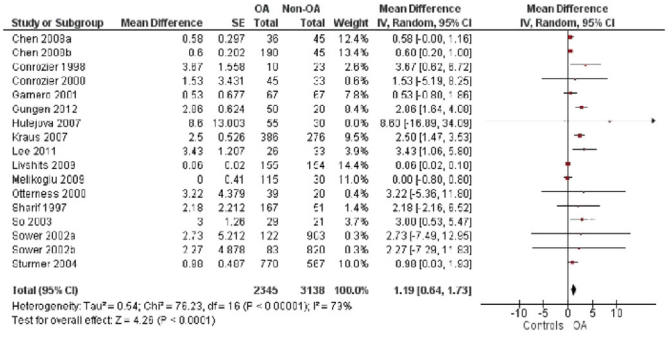

Fig. 1. Comparison of hs-CRP levels between OA and non-OA

511
OLIGOMYCIN, AN INHIBITOR OF COMPLEX V OF THE MITOCHONDRIAL RESPIRATORY CHAIN, INDUCES AN INFLAMMATORY AND OXIDATIVE RESPONSE IN RAT KNEE JOINT.

C. Vaamonde-García †, J. Loureiro †, E. López-Peláez ‡, A. Centeno-Cortés ‡, R.R. Riveiro-Naveira †, M.N. Valcárcel-Ares †, F.J. Blanco §, M.J. López-Armada †. † *Aging and Inflammation Res. Lab, INIBIC - CHU A Coruña, A CORUÑA, Spain;* ‡ *Experimental Surgery Unit, CHU A Coruña, A CORUÑA, Spain;* § *Osteoarticular and Aging Res.Lab. CIBER-BBN. INIBIC- Univ. of A Coruña,, A CORUÑA, Spain*

Purpose: Inflammation is now recognized as a pivotal player in articular damage pathways in OA. A decline of mitochondrial function has been described in OA chondrocytes and RA synoviocytes. Recent ex vivo findings support a connection between mitochondrial dysfunction and activation of inflammatory and destructive pathways in these cells. The aim of this study was to investigate in vivo whether the intraarticular injection of oligomycin (OLI), an inhibitor of mitochondrial function, induces a destructive, oxidative and inflammatory response in rat knee joints.
Methods: 45 female wistar rats (180–220 g) were randomized into three study groups: Healthy (no intraarticular injection); Lipopolysaccharide (LPS)-treated, positive control (left joint injected with 10 μg LPS); and OLI-treated (left joint injected with 20 μg OLI). Right joints were treated with the respective vehicles. Intraarticular injections were carried out on day 0, 2 and 5, and rats were euthanized on day 6. Hind paws were collected and joint tissues were obtained. Measurement of joint diameters on stimulis- and control-injected paws was performed on days 0 and 6. Histopathologic lesions were evaluated by Hematoxilyn-Eosin (H&E) and Masson Trichromic Staining in synovial tissue and by Safranin O staining in cartilage. ROS production was